Kidney International, Vol 21 (1982), pp. 387–394

Glomerular localization of circulating antiglobulin activity in essential mixed cryoglobulinemia with glomerulonephritis

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Glomerular localization of circulating antiglobulin activity in essential mixed cryoglobulinemia with glomerulonephritis. Kidney biopsy samples from 27 patients with essential mixed cryoglobulinemia of the IgG-IgM(*k*) type and glomerulonephritis were studied to assess whether glomerular immunodeposits display antiglobulin (AG) activity similar to that of serum cryo-IgM. A preparation of heat-aggregated human IgG (FAIgG) was used to search for tissue AG activity, and blocking tests and reactivity tests were carried out to define the nature of this activity. Glomerular localization of FAIgG was observed in 17 out of 27 kidney specimens, the positive findings being always associated with IgM deposits. Prior exposure of tissue sections to anti-IgM serum blocked the FAIgG reaction, but no such effect was produced by the pretreatment with other antisera. The positive FAIgG tissue specimens yielded a similar fluorescence pattern with aggregated alkylated-reduced IgG, but did not react at all with the aggregated $F(ab')_2$ or aggregated albumin. The IgM recovered in the eluate of a kidney biopsy specimen displayed AG activity. Patients with AG deposits showed more severe histologic changes and a greater renal functional impairment than did those without. The data support the notion that circulating cryo-IgM anti-IgG participates in the formation of glomerular immunodeposits and in the genesis of renal damage.

Localisation glomérulaire de l'activité circulante antiglobuline au cours de la cryoglobulinémie mixte essentielle avec glomérulonéphrite. Les biopsies rénales de 27 malades atteints de cryoglobulinémie mixte essentielle du type IgG-IgM(k) et de glomérulonéphrite ont été étudiées estimer si les dépôts glomérulaires immuns ont une activité antiglobuline (AG) semblable à celle de la cryo-IgM sérique. Une préparation d'IgG humaine agrégée par la chaleur (FAIgG) a été utilisée pour étudier l'activité AG du tissu alors que des tests bloquants et destests de réactivité ont été réalisés la nature de cette activité. Une localisation glomérulaire de FAIgG a été observée dans 17 parmi les 27 échantillons de rein, cette localisation a toujours été associée à des dépôts d'IgM. L'exposition préalable des coupes à un sérum anti-IgM a bloqué la réaction de FAIgG mais cet effet n'a pas été produit par le prétraitement au moyen d'un autre antisérum. Les échantillons positifs pour FAIgG ont donné une image en fluorescence semblable avec de l'IgG agrégée à activité réduite mais n'ont pas réagi avec $F(ab')_2$ agrégée ou l'albumine agrégée. L'IgM obtenue par élution d'un échantillon de biopsie rénale a montré une activité AG. Les malades porteurs de dépôts d'AG avaient des modifications histologiques plus importantes et une altération de la fonction rénale plus grande que ceux qui en étaient dépourvus. Ces observations sont en faveur de la notion selon laquelle la cryo-IgM circulante anti-IgG participe à la formation de dépôts immuns glomérulaires et à la genèse de lésions rénales.

An immune complex mechanism likely sustains the renal involvement that occurs in 20 to 50% of patients with essential mixed cryoglobulinemia (EMC). Indeed, the circulating cryoglobulins themselves are strongly suspected as the offending immune reactants [1–4].

This work was undertaken to provide further support to the hypothesis that the circulating cryoglobulins are involved in the genesis of glomerulonephritis through their deposition in the glomeruli. We thought that if the serum cryoglobulins participate in the formation of glomerular immunodeposits, these latter should contain a moiety displaying antiglobulin (AG) activity in the same way as one cryoglobulin component, usually the IgM, does. Hence, we tried to assess whether (*a*) the glomerular immunodeposits show AG activity and, if so, whether (*b*) this activity derives from circulating cryo-IgM, and (*c*) bears some relationship with the degree of renal damage.

Methods

Patients. The study was carried out on 27 patients with EMC who had been admitted during the years 1975 through 1980 to any one of the four renal units participating in this study. Diagnosis of EMC was established after having excluded lymphoproliferative disorders and other primary diseases known to cause cryoglobulinemia, such as SLE, acute poststreptococcal glomerulonephritis, and so on. None of them had HBs antigenemia. All patients showed kidney involvement manifested by pathologic urinary findings (proteinuria and/or microhematuria), mild to severe impairment in renal function, and consistent histologic changes on renal biopsy samples. Other system involvement and the relevant data of these patients are reported in Table 1.

Cryocrit values of sera collected according to the recommendations of Grey and Köhler [2] ranged between 2% and 20%. Analysis of the cryoprecipitates, carried out in the Center of Clinical Physiology (CCP) on sera stored at −20° C, according to previously reported methods [2], showed that serum cryoglobulins were composed of IgG-IgM(*k*) in 24, and of IgG-IgM(*k*)-C1q in the remaining 3 patients (no. 4, 11, 27). Serum AG activity, assessed by latex agglutination, ranged in reciprocal

Received for publication February 25, 1981
and in revised form August 17, 1981

0085-2538/82/0021-0387 $01.60

Table 1. Clinical and laboratory data[a]

Patient no.	Age/ sex	MAP *mm Hg*	S_{Cr} *mg/dl*	$U_{prot.}$ *g/24 hr*	Cryocrit %	C3 *mg/dl*	C4 *mg/dl*	C1q %	Serum AG titer[b]	Other findings
1	48/M	126	1.4	3.0	14	35	2	34	2.0	CAH, paresthesias, leg ulcers
2	57/F	123	2.0	2.0	8	35	§	78	1.6	HML, paresthesias, leg ulcers
3	54/F	120	1.5	2.0	7	65	4	45	0.3	Hepatosplenomegaly
4	53/M	103	1.1	0.5	2	80	6	ND	0.2	CAH
5	50/M	133	2.0	8.5	4	30	15	72	0.8	Hepatosplenomegaly
6	50/M	106	1.3	1.5	5	17	9	20	1.4	CAH
7	68/M	123	1.9	0.7	3	25	§	8	0.2	—
8	60/M	121	3.0	1.0	11	15	§	§	0.3	Hepatomegaly
9	45/M	123	2.2	5.2	3	63	§	§	0.3	Hepatosplenomegaly
10	64/M	126	2.3	1.5	16	42	4	13	0.2	Paresthesias, leg ulcers
11	51/F	126	1.5	2.9	9	60	30	ND	0.2	Hepatomegaly
12	53/F	113	0.7	2.0	17	72	4	ND	0.1	Hepatomegaly
13	65/M	106	1.7	2.6	16	50	ND	ND	0.3	Hepatomegaly, Raynaud's phenomenon
14	54/F	116	3.0	2.5	16	45	10	50	0.6	Hepatosplenomegaly, leg ulcers
15	52/M	103	0.8	0.4	5	54	5	ND	0.3	Hepatomegaly
16	43/M	126	2.0	15.0	20	31	5	ND	1.3	Hepatomegaly
17	52/M	133	1.8	3.0	17	55	40	40	0.6	Hepatomegaly
18	46/M	96	1.0	0.4	6	45	56	60	4.0	Hepatomegaly
19	56/F	150	1.8	0.7	18	36	12	100	5.0	Hepatomegaly, paresthesias, Raynaud's phenomenon
20	48/M	133	1.5	0.0	5	69	7	82	0.6	Hepatomegaly
21	44/M	110	0.8	7.0	4	61	3	76	0.3	—
22	46/F	133	1.8	9.0	2	55	21	77	0.2	Hepatomegaly
23	36/F	123	1.6	5.0	8	95	50	70	0.3	Hepatomegaly
24	64/F	123	1.1	0.1	3	70	36	ND	0.6	—
25	51/F	96	0.6	2.0	15	90	4	ND	0.6	Hepatomegaly
26	55/F	103	0.7	0.6	7	55	4	ND	4.0	Leg ulcers, Raynaud's phenomenon
27	53/M	106	1.6	0.1	2	80	15	78	1.6	CAH

[a] Abbreviations are defined as follows: S_{Cr}, serum creatinine; $U_{prot.}$, urinary proteins; MAP, mean arterial pressure; §, undetectable; ND, not done; CAH, chronic active hepatitis; HML, hepatic minimal lesions.

[b] Measured as reciprocal $\times 10^3$.

titer from 100 to 5000 and was almost entirely (90% or more) cryoprecipitable. Twenty-five patients had reduced serum levels of one or more of the three complement factors (C3, C4, C1q) that were routinely assayed by radial immunodiffusion.

Kidney tissue specimens, obtained in each of the 27 patients by either percutaneous (23 patients) or open biopsy (4 patients), were processed for light and immunofluorescence microscopy. Each specimen contained from 5 to more than 60 glomeruli, with most of them containing 10 to 20 glomeruli.

Immunofluorescence. The fluorescence studies were carried out by an independent observer (FB) on tissue that had been stored at −80° C. The frozen kidney slices were tested by the direct immunofluorescence technique with rabbit monospecific fluorescein-labeled antisera against human IgM, IgG, IgA, C3, C4, C1q, light chain of either kappa and lambda type, fibrinogen, and albumin. For the C3 activator (C3A), the indirect fluorescence technique was used. All these antisera were purchased from Behringwerke.

The assay for tissue AG activity was carried out on unfixed sections with a solution of fluoresceinated aggregated human IgG (FAIgG) prepared from commercial Cohn fraction II according to the method of Rossen et al [5]. The specificity control of the reaction with the FAIgG was assessed by treating the tissue kidney sections with a solution of unfluoresceinated AIgG having the same concentration as the labeled preparation (3 mg/ml). A fluoresceinated preparation of native human IgG (3.2 mg/ml), obtained from the same batch of commercial Cohn fraction II, was also used for the immunofluorescence testing; before use, this preparation was purified through Sepharose 6B chromatography to remove the aggregated molecules.

To identify the nature of the tissue deposits endowed with AG activity, we carried out blocking experiments as outlined by Rossen et al [6]: the unfixed frozen sections were treated with unconjugated antihuman IgG, IgA, IgM, and C1q sera, washed with 0.01 M phosphate buffer saline (pH, 7.2) (PBS), and then exposed to the FAIgG reagent.

Further studies included the use of fluoresceinated solutions of aggregated alkylated and reduced IgG (A/R FAIgG) (3 mg/ml), and of aggregated $F(ab')_2$ (3.5 mg/ml), both obtained from human Cohn fraction II, and aggregated human albumin (3.5 mg/ml). These reagents were prepared according to the methods outlined by Dikler [7], stored at −80° C, and used within 1 month of their preparation.

In an attempt to reveal blocked AG activity on cryostat sections, we used partial elution techniques as described by Koffler, Schur, and Kunkel [8]. Before direct immunofluorescence testing for IgG, IgM, and AG activity was performed, selected cryostat sections were treated with 0.02 M citrate buffer (pH, 3.2) for periods of 30 min to 12 hours; 1 M or 2 M sodium chloride for periods of 30 to 120 min; 1 M or 2 M KI for periods of 30 to 120 min. All treatments were carried out at room temperature. As control, tissue sections were exposed to PBS for comparable periods of time.

Elution studies. A surgical specimen of kidney cortex weighing 0.70 g was minced finely, suspended in 15 ml of PBS, and homogenized in a chilled Waring Blendor for 2 min. The

homogenate was washed repeatedly with 30-ml aliquots of PBS by cold centrifugation at $\times 15000g$ for 20 min. After 20 washings, the supernatant, which had been concentrated 30-fold in a cellophane bag against PEG 6000, contained no protein detectable at 280-nm spectrophotometric analysis. The pellet was suspended in 14 ml of 0.2 M glycine buffer (pH, 2.4) and stirred for 4 hours at room temperature, then incubated overnight at 4° C. After centrifugation, the eluate was dialyzed against 0.1 M acetate buffer (pH, 4.0), concentrated to the final volume of 2 ml, and then fractionated on Sephadex G-200 chromatography at 4° C in 0.1 M acetate buffer (pH, 4.0). Two-milliliter fractions were collected, and their optical density was determined on a Perkin-Elmer Coleman III spectrophotometer at 280 nm. The protein fractions obtained were dialyzed against PBS, concentrated, and tested for serum proteins by double immunodiffusion on agar plates with rabbit antisera (Behringwerke) to human serum, C1q, IgG, IgA, IgM, L chains type kappa and lambda, and albumin. Antisera were put in the peripheral wells (4 mm in diameter) and allowed to diffuse for 24 hours before twice filling the central well (8 mm in diameter) with the sample to be tested. The IgG and IgM were quantified by a Behring laser nephelometer.

Light microscopy. The sections stained with hematoxylin-eosin, periodic acid-Shiff reagent, Masson's thrichrome, and silver methenamine were examined by an observer (GBB) to whom the results of the immunofluorescence examination were not known. He graded the histologic activity and the severity of the lesions semiquantitatively from 0 to 3+, taking into account the following features: endocapillary hypercellularity (endothelial and mesangial), number of polymorphonuclear leucocytes, epithelial crescents, intraluminal "thrombi," and interstitial cellular infiltration. The finding of vasculitis was scored 3+. Overall renal histologic activity was evaluated on the basis of the severity scores of all these changes.

Statistical analysis. The data were analyzed with the χ^2 test with Yates' correction and the two-tailed Wilcoxon rank sum test. The 0.05 level of significance was used to determine statistically significant results.

Results

Tissue AG activity. The immunofluorescence findings in the patients with EMC are shown in Table 2. Of the 27 kidney biopsy samples examined, 26 had glomerular deposits of IgM, 24 of IgG, 17 of C3, and 12 of other complement factors of either the classical or the alternative pathway. The appearance of the fluorescent staining was similar to that previously reported by others [1–4, 9–13].

Seventeen biopsy specimens stained with the FAIgG (Fig. 1A) in a specific fashion, as documented by the blocking effect of previous treatment with the unfluoresceinated AIgG. The tissue staining, which had a granular appearance and sometimes filled the capillary lumina, was located in every instance at sites containing IgM deposits. But several kidney specimens, though loaded with IgM deposits, did not react with the FAIgG. In six instances, positive staining for FAIgG was observed in tissue specimens not containing deposits of complement factors (Table 2).

The treatment with A/R FAIgG yielded results quite similar to those observed with FAIgG (Table 3 and Fig. 1B) in the 15 patients in whom enough tissue was left to carry out this test. Moreover, prior incubation of tissue sections with unfluoresceinated alkylated and reduced aggregated IgG inhibited the subsequent staining with the FAIgG, suggesting the same tissue binding sites were implicated in both reactions. None of the FAIgG positive specimens stained with the aggregated fluoresceinated $F(ab')_2$ (Fig. 1C) or the aggregated human albumin preparations, and only 1 out of 10 bound the native human IgG (Table 3).

The AG positive tissue specimen with isolated IgM deposits (patient 4) stained only for kappa chains when tested with the specific antisera for both light chains, suggesting that IgM deposits were of monoclonal type (Fig. 2, A–D).

In all patients with renal deposits of AG activity, pretreatment of frozen kidney sections with unconjugated anti-IgM serum blocked the subsequent reaction with the FAIgG (Fig. 1D). By contrast, the pretreatment with unconjugated antisera against IgG, IgA or C1q had no blocking effect on the FAIgG staining.

Treatment with salt or acid solutions appeared to affect both the IgG and the IgM deposits in much the same way. Of 5 FAIgG-negative specimens examined, none was converted into positive by the elution procedures, whether by those bringing about the complete removal of immunoglobulin deposits (0.02 M citrate buffer pH 3.2 for 2 hours, 2 M sodium chloride for 30 min, 2 M KI for 2 hours) or by those leaving some amounts of them in the tissue (1 M sodium chloride for 30 min; 1 M KI for 30 min). The 8 FAIgG-positive specimens lost their FAIgG reactivity after IgM deposits had been completely or nearly completely removed by acid or salt solutions. PBS treatment for comparable periods of time caused only a negligible reduction in the intensity of the immunofluorescent staining.

Characterization of the kidney eluate. On Sephadex G-200 chromatography, the acid eluate of the open-kidney biopsy sample obtained from patient 6 yielded one very small protein peak only, which was eluted with the void volume. When tested with the panel of antisera, this protein fraction appeared to contain IgM solely. Total amount of IgM recovered was 24 μg. All the remaining effluent fractions, after being pooled, dialyzed, and concentrated, reacted only with the anti-IgG serum in the Ouchterlony plate. Total amount of IgG recovered was 33 μg. On further testing, the IgG fraction gave precipitin lines with both the anti-kappa and anti-lambda sera, whereas the IgM fraction reacted with the anti-kappa serum only (Fig. 3). But because the amount available for testing was at the limit of the sensitivity of the technique for polyclonal IgM, we cannot exclude its presence in the eluate. At the latex test, only the IgM fraction displayed AG activity, at a titer per unit of weight similar to that of serum cryo-IgM (Table 4).

Tissue AG activity and renal damage. When grouped according to the presence (group 1, 17 patients) or absence (group 2, 10 patients) of AG activity in their biopsy specimens, the patients did not differ with regard to the prevalence of positive staining for IgG, IgM, IgA, and C3. But 9 and 3 patients of group 1 had deposits of C1q and C4, respectively, whereas only 1 patient in group 2 had deposits of C1q (Table 2). The histologic changes observed at light microscopy in the two groups are compared in Table 5. Overall histologic activity averaged 8.8 ± (SD) 2.7 in group 1 and 4.2 ± 2.9 in group 2, a highly significant difference

Table 2. Renal histologic pattern and immunofluorescence findings[a]

Patient no.	Histology	Reactivity with FAIgG	Glomerular deposits						
			IgG	IgM	IgA	C3	C4	C1q	C3A
1	MPGN	++T	+++	+++T	++	++	++	++	+
2	MPGN	+	++	++	−	−	−	−	−
3	MPGN	+	++	++	+	+	+	+	+
4	MsPGN	++	−	++	−	−	−	−	−
5	MPGN	++	+	+	−	−	−	−	−
6	MPGN	+++	+++	+++TA	++	+	−	+	ND
7	MPGN	++T	++	+++T	++	−	−	−	−
8	MPGN	++	+++	+++TA	+++	++	−	+	−
9	MPGN	+	++	+++	−	+	−	−	ND
10	MPGN	++	+++	+++	+	+	−	+	ND
11	MPGN	++T	+	+T	−	−	−	−	−
12	MPGN	+++	++	+++	+	+	−	−	ND
13	MPGN	+	+++	+++	−	+	+	+	+
14	MPGN	+++	+++	+++TA	++	+	−	+	+
15	MsPGN	++	++	+	−	−	−	−	−
16	MPGN	++	++	++	−	−	−	+	+
17	MPGN	+	+++	++	+	+	−	+A	−
Total		*17*	*16*	*17*	*9*	*10*	*3*	*9*	*5*
18	MPGN	−	+	++A	−	−	−	+	−
19	MPGN	−	+++	+++	−	−A	−	−	−
20	MsPGN	−	+	++	+	++	−	−	−
21	MPGN	−	+	+	−	+	−	−	−
22	MPGN	−	+	++	+	+	−	−	−
23	MPGN	−	+	++T	−	+++	−	−	+
24	Vasculitis	−	−A	−A	−A	−A	−	−A	ND
25	MPGN	−	++	++	−	+	−	−	−
26	FPGN	−	+	+	−	++	−	−	+
27	MsPGN	−	−	+	−	+	−	−	−
Total		*0*	*8*	*9*	*2*	*7*	*0*	*1*	*2*

[a] Abbreviations are defined as follows: MPGN, membranoproliferative glomerulonephritis; MsPGN, mesangioproliferative glomerulonephritis; FPGN, focal proliferative glomerulonephritis; T and A, concomitant tubular and arterial deposits, respectively.

($P < 0.002$) due to the higher scores of endocapillary hypercellularity, granulocyte infiltration, intraluminal "thrombi," and vasculitis in group 1. Compared with controls, both groups had abnormally low serum levels of C3, C4, and C1q ($P < 0.01$ for all three variables), but in group 1 serum levels of C3 and C1q were significantly lower, and creatinine serum level significantly higher than they were in group 2 (Table 6). The two groups did not differ significantly as to age, degree of proteinuria, blood pressure, cryocrit, and serum AG activity titer.

Discussion

So far, the evidence supporting the view that serum cryoglobulins participate in the formation of glomerular immunodeposits in EMC glomerulonephritis is indirect, it being based on the following observations: (*1*) The mixed cryoglobulins are similar to immune complexes because they are usually made of a monoclonal IgM anti-IgG and a polyclonal IgG that react with each other by precipitating at cold temperature [1–3]. (*2*) The immunoglobulin classes of the cryoprecipitates are nearly always represented within the renal immmunodeposits [1–4, 9–13]. (*3*) At electron microscopy examination, the cryoprecipitates show a fibrillar appearance closely resembling that of the subendothelial deposits of the glomeruli [10, 14].

Extending our previous observations [15, 16], this study shows that kidney tissue from the majority (17 out of 27) of patients with EMC glomerulonephritis contains, like their serum cryoprecipitates, some factor(s) that specifically bind(s) in vitro the aggregated IgG. Such a prevalance of positive stainings for the FAIgG is restricted to EMC glomerulonephritis and a few other forms of glomerulonephritis. In fact, in this laboratory (CCP), where, since 1978, all kidney biopsy samples are routinely tested for AG activity, we have so far detected the AG deposits in only 11 out of the 98 biopsy examinations performed on patients with a wide variety of glomerulonephritis other than that associated to EMC. In line with other reports [5, 6, 17, 18], the positive findings were limited to 3 of 14 patients with SLE nephritis, 6 of 14 with acute poststreptococcal glomerulonephritis, and 2 of 3 with proliferative extracapillary glomerulonephritis.

We collected several bits of evidence pointing out that tissue AG activity derives from the circulating cryo-IgM:

(*1*) In all EMC patients, the serum cryoglobulin component having AG activity was an IgM(k), and the IgM was the only moiety that we consistently detected in association with the AG activity within the glomeruli.

(*2*) The tissue specimens with positive FAIgG reaction reacted also with the A/R FAIgG, but failed to bind the fluoresceinated aggregated $F(ab')_2$ and the aggregated albumin reagents. Such a reactivity profile pertains also to the circulating cryo-IgM anti-IgG [19]. In keeping with IgM anti-IgG's affinity of 100

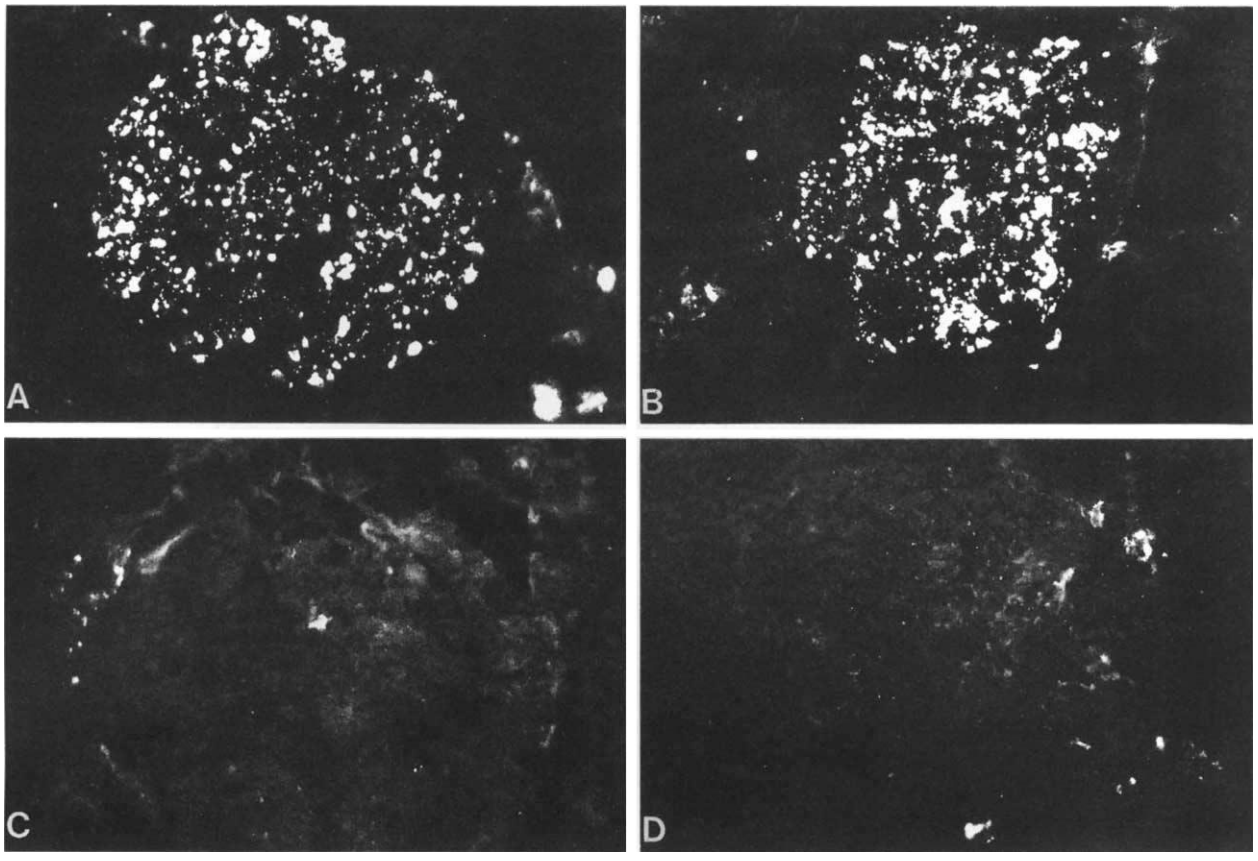

Fig. 1. *Serial cryostat sections of a glomerulus incubated with* **A** *fluoresceinated aggregated human IgG (FAIgG),* **B** *alkylated and reduced FAIgG,* **C** *fluoresceinated F(ab')$_2$,* **D** *unfluoresceinated anti-IgM serum followed by FAIgG.* Scattered tissue staining outside the glomerulus is due to autofluorescence or serum precipitates (×245).

Table 3. Association of FAIgG binding with immunofluorescence reactions with A/R FAIgG, aggregated F(ab')$_2$, aggregated albumin, and native IgG

Reactivity with FAIgG	No. of patients	A/R FAIgG[a] *no. positive*	Aggr. F(ab')$_2$ *no. positive*	Aggr. albumin *no. positive*	Native IgG *no. positive*
Positive	10	10	0	0	1
Negative	5	0	0	0	—

[a] A/R FAIgG is alkylated and reduced fluoresceinated aggregated IgG.

times greater for the aggregated IgG than for the monomeric IgG [20], in 9 over 10 patients the AG deposits did not react with the monomeric native human IgG.

(*3*) The pretreatment of tissue sections with the anti-IgM unfluoresceinated serum blocked the reaction with the FAIgG, whereas the pretreatment with the unfluoresceinated anti-IgG, anti-IgA, or anti-C1q sera had no such effect. A reactivity pattern like this strongly suggests that the IgM deposits sustain tissue AG activity [6]. The alternative explanation that the FAIgG tissue staining could be sustained by either the Fc receptors of tissue and/or inflammatory cells (macrophagi and other) or C1q deposits appears untenable. In fact, as against the rheumatoid factor, neither Fc receptors nor C1q bind the alkylated and reduced aggregated IgG [7, 21, 22].

(*4*) The observation that on specific testing the isolated IgM glomerular deposits[1] in one patient appeared of monoclonal kappa type like his circulating cryo-IgM also supports the notion that this serum cryoglobulin component was the source of the tissue AG activity.

(*5*) After purification in acid medium so as to prevent its reaggregation with IgG, the IgM present in the eluate of a kidney biopsy, on which immunofluorescence studies had demonstrated deposits of IgM and AG activity within the glomeruli, displayed antiglobulin activity with nearly the same titer as did the autologous serum cryo-IgM(*k*).

[1] The occurrence of isolated IgM deposits has been reported by others [10] in patients with EMC and glomerulonephritis.

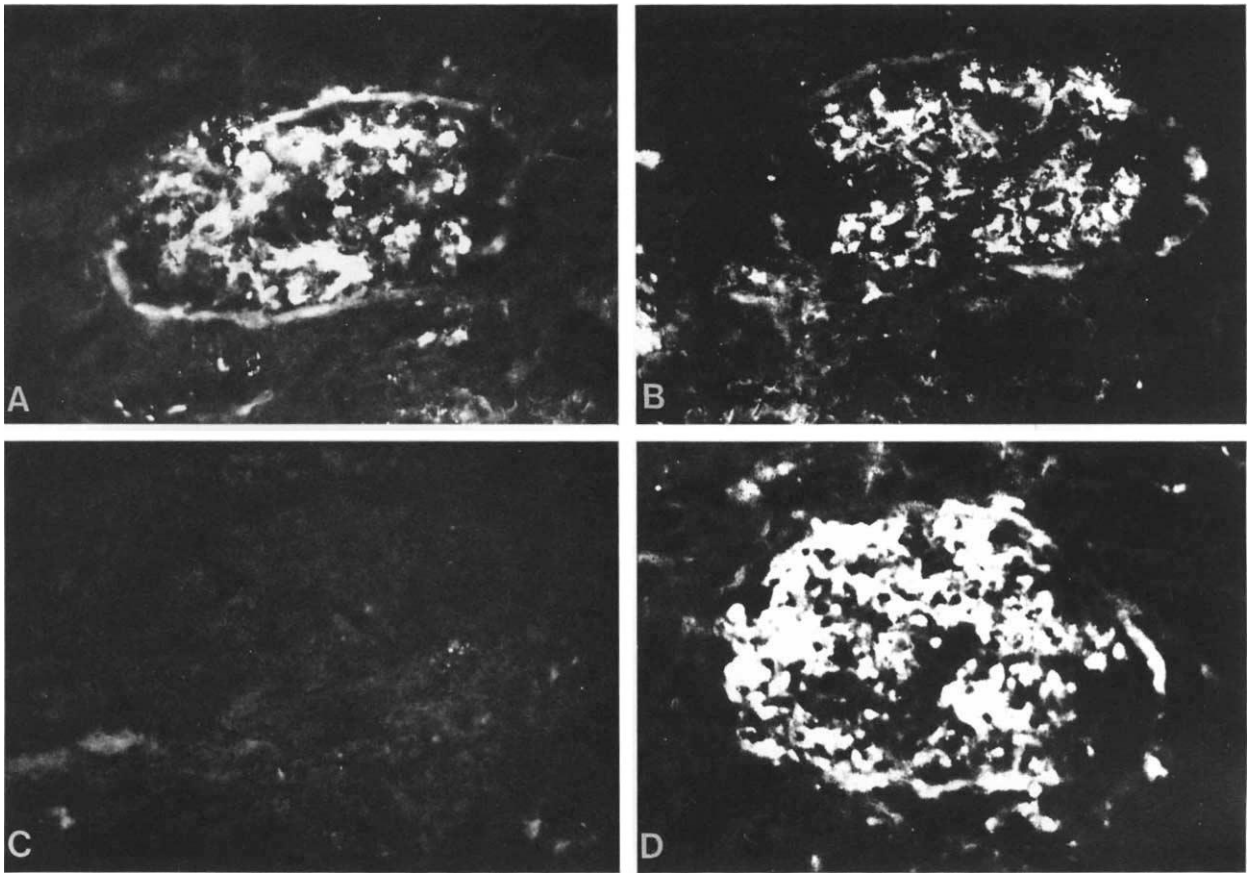

Fig. 2. *Serial cryostat sections of a glomerulus (patient 4) incubated with* **A** *fluoresceinated anti-IgM,* **B** *fluoresceinated anti-kappa chain,* **C** *fluoresceinated anti-lambda chain,* **D** *FAIgG.* Scattered tissue staining outside the glomerulus is due to autofluorescence or serum precipitates (×245).

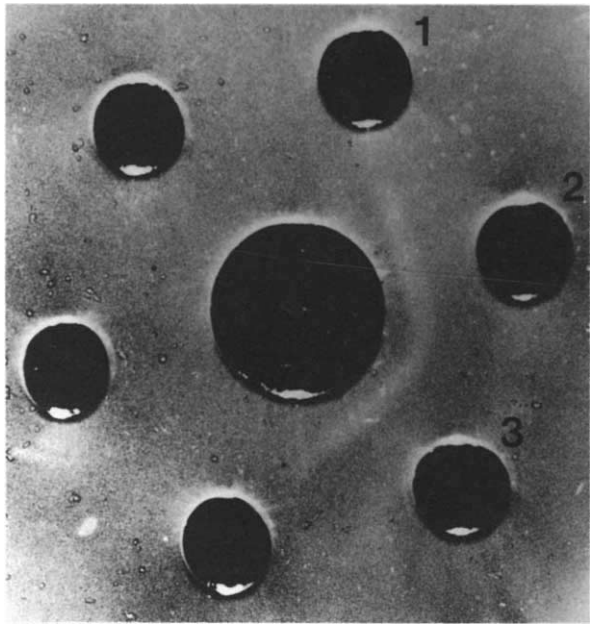

Fig. 3. *Double diffusion plate.* The IgM fraction of the kidney eluate *(central well)* reacts with both anti-IgM serum *(well 2)* and anti-kappa serum *(well 3).* No reaction occurred with anti-lambda serum *(well 1).*

Table 4. Characterization of kidney biopsy eluate in patient 6

	Serum supernatant	Serum cryoglobulins	Kidney eluate
IgG, *mg/dl*	350.0	250	3.3
IgM, *mg/dl*	155.0	120	2.4
Ag activity, *titer reciprocal*	40.0	1400	24.0
AG activity:IgM	0.2	11	10.0

Taken together, the above observations provide compelling evidence that in patients with EMC glomerulonephritis renal AG activity derives from circulating cryo-IgM anti-IgG. Previously, Agnello, Koffler, and Kunkel [17] had provided direct evidence for glomerular localization of circulating IgM anti-IgG in SLE nephritis using fluoresceinated idiotypic antiserum.

In a substantial proportion of patients, 10 over 27, the glomerular IgM deposits did not display any AG activity. These negative findings, however, might have been due to the saturation of binding sites preventing their reaction with the aggregated IgG. Attempts at unmasking blocked AG activity by procedures of partial elution were inconclusive because, as it occurs with other immune complex systems [23], each eluting agent removed in parallel both IgG and IgM deposits from the

Table 5. Kidney histopathology in patients with and without tissue antiglobulin activity

Elementary lesions	Reactivity with FAIgG: Positive (N = 17)	Reactivity with FAIgG: Negative (N = 10)	X^2_c	P
Endocapillary hypercellularity ≥ 2+	13	3	3.87	<0.05
Polymorphonuclear leucocytes infiltration	12	2	4.58	<0.05
Epithelial crescents	8	2	0.98	NS
Intraluminal thrombi	12	2	4.58	<0.05
Interstitial infiltration ≥ 2+	10	3	1.09	NS
Vasculitis	12	2	4.58	<0.05

Table 6. Comparison of patients with and without tissue antiglobulin activity

Data	Reactivity with FAIgG[a]: Positive (N = 17)	Reactivity with FAIgG[a]: Negative (N = 10)	P[b]
Age, *yr*	54.0 ± 7.0	50.0 ± 8.0	NS
MAP, *mm Hg*	119.0 ± 10.0	117.0 ± 18.0	NS
Serum creatinine, *mg/dl*	1.8 ± 0.6	1.2 ± 0.4	<0.04
Urine proteins, *g/24 hr*	3.2 ± 3.6	2.5 ± 3.3	NS
Cryocrit, *%*	10.0 ± 6.0	7.0 ± 5.0	NS
Serum AG, *titer reciprocal* × 10^3	0.6 ± 0.5	1.7 ± 1.8	NS
Serum C3[c], *mg/dl*	45.0 ± 19.0	65.0 ± 19.0	<0.02
Serum C4[c], *mg/dl*	8.0 ± 11.0	21.0 ± 20.0	NS
Serum C1q[c], *% normal values*	32.7 ± 27.7	77.5 ± 12.1	<0.003

[a] Values are the means ± SD (N = 50).
[b] By Wilcoxon rank sum test.
[c] Normal values (mean ± SD): C3, 90.7 ± 21.0; C4, 35.0 ± 7.4; C1q, 98.2 ± 16.6.

cryostat sections. Moreover, it is possible that AG activity was present at such low levels as to escape the detection system we used. Studies on kidney specimens large enough to allow elution and characterization of their IgM deposits would probably solve these uncertainties.

We observed that patients with demonstrable glomerular deposits of AG activity had evidence of more severe histologic renal lesions, lower serum levels of C3 and C1q, and greater impairment in renal function than did those without. These observations, however, although in keeping with previous reports on other forms of glomerulonephritis [5, 6], do not provide an answer as to whether the glomerular-entrapped AG activity plays a pathogenic role or is merely a consequence of the glomerular damage. We favor the former hypothesis because the marked reduction or the disappearance of the circulating cryo-IgM anti-IgG, induced by treatment [24–26], results in the marked improvement of glomerulonephritis.

Why and how circulating AG gets lodged in the glomeruli in only a proportion of the patients with EMC also remain to be elucidated. Whatever the mechanism might be, our in vitro studies showed that the deposits of AG were undersaturated in their binding capacity of the aggregated IgG. Thus, the glomeruli of these patients contained a moiety which, at least in vitro, is known to act as an immune reactant capable of binding the aggregated IgG, monomeric IgG, and immune complexes of widely varying size [20]. It is tempting to speculate that such deposits act even in vivo as an amplifying mechanism of the glomerular localization of the circulating immune complexes and/or of other immune reactants. This hypothesis would account for the plentifulness of glomerular immunodeposits so often observed in EMC glomerulonephritis. Because AG activity has been detected in the kidney of patients with other forms of immunologically mediated renal disease [5, 6, 17, 18], this mechanism might be of much greater importance than previously thought.

Acknowledgments

Part of this work has been presented at the 8th International Congress of Nephrology, Athens, June 7–12, 1981. Mr. N. Marino gave technical assistance.

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References

1. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N: Cryoglobulinemia: A clinical and laboratory study: II. Cryoglobulins with Rheumatoid Factor activity. *Am J Med* 40:837–856, 1966
2. Grey HM, Köhler PF: Cryoimmunoglobulins. *Sem Hematol* 10:87–112, 1973
3. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M: Biologic and clinical significance of cryoglobulins: A report of 86 cases. *Am J Med* 57:775–788, 1974
4. Gorevic PD, Kassab HJ, Levo Y, Köhn R, Meltzer M, Prose P, Franklin EC: Mixed cryoglobulinemia: Clinical aspects and long-term follow-up of 40 patients. *Am J Med* 69:287–308, 1980
5. Rossen RD, Rickaway RH, Reisberg MA, Singer DB, Schloeder FX, Suki WN, Hill LL, Eknoyan G: Renal localization of antiglobulins in glomerulonephritis and after renal transplantation. *Arthritis Rheum* 20:947–961, 1977
6. Rossen RD, Reisberg MA, Sharp JT, Suki WN, Schloeder FX, Hill LL, Eknoyan G: Antiglobulins and glomerulonephritis: Classification of patients by the reactivity of their sera and renal tissue with aggregated and native human IgG. *J Clin Invest* 56:427–437, 1975
7. Dikler HB: Studies of the human lymphocyte receptor for heat-aggregated or antigen-complexed immunoglobulin. *J Exp Med* 140:508–522, 1974
8. Koffler D, Schur PH, Kunkel HG: Immunological studies concerning the nephritis of systemic lupus erythematosus. *J Exp Med* 126:607–624, 1968
9. Verroust P, Mery JP, Morel-Maroger L, Clauvel JP, Richet G: Les lesions glomerulaires des gammopathies monoclonales et des cryoglobulinemies idiopathiques IgG-IgM, in *Actualitè Nephrologique de l'Hôpital Neckèr*. Paris, Flammarion, 1971, pp 167–202
10. Feiner H, Gallo G: Ultrastructure in glomerulonephritis associated with cryoglobulinemia: A report of six cases and review of the literature. *Am J Pathol* 88:145–162, 1977
11. Feizi T, Gitlin N: Immune complex disease of the kidney

associated with chronic hepatitis and cryoglobulinemia. *Lancet* 2:873–876, 1969

12. DRUET P, LETONTURIER P, CONTET A, MANDET C: Cryoglobulinemia in human renal disease: A study of seventy-six cases. *Clin Exp Immunol* 15:483–496, 1973
13. ZIMMERMAN SW, DREHER WH, BURKHOLDER PM, GOLDFARB S, WEINSTEIN AB: Nephropathy and mixed cryoglobulinemia: Evidence for an immune complex pathogenesis. *Nephron* 16:103–115, 1976
14. CORDONNIER D, MARTIN H, GROSLAMBERT P, MICOUIN C, CHENAIS F, STOEBNER P: Mixed IgG-IgM cryoglobulinemia with glomerulonephritis: Immunochemical, fluorescent and ultrastructure study and in vitro cryoprecipitate. *Am J Med* 59:867–872, 1975
15. MAGGIORE Q, BARTOLOMEO F, MISEFARI V, L'ABBATE A, MARTORANO C: Role of HBsAg immune complexes in glomerulonephritis of essential mixed cryoglobulinemia, in *Proc 26th Colloquium, 1978, on Protides of the Biological Fluids,* edited by PEETERS H, Oxford, New York, Pergamon Press, 1979, pp 283–286
16. MAGGIORE Q, BARTOLOMEO F, L'ABBATE A, MISEFARI V: HBsAg glomerular deposits in glomerulonephritis: Fact or artifact? *Kidney Int* 19:579–586, 1981
17. AGNELLO V, KOFFLER D, KUNKEL HG: Immune complex systems in the nephritis of systemic lupus erythematosus. *Kidney Int* 3:90–99, 1973
18. MCINTOSH RM, GARCIA R, RUBIO L, RABIDEAU D, ALLEN JE, CARR RI, RODRIGUEZ-ITURBE B: Evidence for an autologous immune complex pathogenic mechanism in acute poststreptococcal glomerulonephritis. *Kidney Int* 14:501–510, 1978
19. JOHNSTON SL, ABRAHAM GN: Studies of human IgM anti-IgG cryoglobulins: I. Patterns of reactivity with autologous and isologous human IgG and its subunits. *Immunology* 36:671–683, 1979
20. GABRIEL A JR, AGNELLO V: Detection of immune complexes. The use of radioimmunoassays with C1q and monoclonal rheumatoid factor. *J Clin Invest* 59:990–1001, 1977
21. MIZOGUCHI Y, TANIMOTO K, YUE C, HORIUCHI Y: Immune receptors (IgG-Fc and complement receptors) in normal human organs. *Lab Invest* 40:703–707, 1979
22. AGNELLO V, WINCHESTER RJ, KUNKEL HG: Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology* 19:909–919, 1970
23. WOODROFFE AJ, WILSON GB: An evaluation of elution techniques in the study of immune complex glomerulonephritis. *J Immunol* 118:1788–1794, 1977
24. RISTOW SC, GRINER PF, ABRAHAM GN, SHOULSON I: Reversal of systemic manifestations of cryoglobulinemia. Treatment with melphalan and prednisone. *Arch Intern Med* 136:467–470, 1976
25. L'ABBATE A, PACIUCCI A, BARTOLOMEO F, MISEFARI V, NOBILE F, CERRAI T, MAGGIORE Q: Selective removal of plasma cryoglobulins in cryoglobulinemia. *Proc Eur Dial Transpl Assoc* 14:486–494, 1977
26. MAGGIORE Q, L'ABBATE A, BARTOLOMEO F, MISEFARI V, CACCAMO A, MARTORANO C: Plasmapheresis and Cryopheresis, in *Advances in Nephro-Urology: Immunology of renal disease,* edited by PAVONE-MACALUSO M, SMITH PH, Plenum Press, New York, London, 1980, in press